REMARKS/ARGUMENTS

Claims 2-4, 7-13 and 16 are active.

Claim 2, the first independent claims defines the invention as follows:

A method for reducing the effect of a fructosyl lysine compound in an assay of a glycated protein-containing sample, the method comprising

treating the sample with a protease to release free fructosyl valine or fructosyl valylhistidine,

reacting a fructosyl peptide oxidase with the released fructosyl valine or fructosyl valylhistidine in the sample at a pH of 4.0 to 7.0 to produce hydrogen peroxide-thereby reducing the effect of fructosyl lysine compound in the assay,

measuring the product of the reacting at a pH of 4.0 to 7.0; and

correlating the measuring of the product to the presence or level of glycated protein in the sample.

As described in the application, reacting a fructosyl peptide oxidase with the released fructosyl valine or fructosyl valylhistidine in the sample at a pH of 4.0 to 7.0 to produce hydrogen peroxide reduces the effect of fructosyl lysine compound in the assay.

Applicants thank the Examiner for the courtesy of discussing this application with their undersigned representative on October 20, 2010 and specifically the rejections based on the previously cited Hirokawa. During this discussion, the Examiner explained that the values of 6.5 and 8.0 in terms of pH in Hirokawa were not being relied upon to teach a range but that a pH 6.5 is a value within the claimed range. It was explained that the pH of 6.5 in Hirokawa is when the sample is treated with protease and only subsequently when that sample is diluted with a buffer at a pH of 8.0 is the oxidase used in the reaction. Further, the Examiner made note of Hirokawa's Figure 1 and the assessment of pH on the activities of the enzymes described but it was explained that the reaction was performed in the absence of

fructosyl lysine as discussed on page 105, col. 1 in the section titled: "Assay of FPOX activity."

In the Interview Summary regarding that meeting, the Examiner has indicated that the anticipation rejection is to be withdrawn. However, as there is an indication that Hirokawa is applicable under 35 USC 103, Applicants explain further below why the claims of the present application would not have been obvious in view of Hirokawa.

Hirokawa treats a sample (Fru-hexapeptide) with an *Aspergillys orzae* protease at a pH of 6.5 and thereafter adds a reaction mixture, including the oxidase, which is buffered at a pH of 8.0 (see page 106, col. 1 "*Enzymatic measurement of glycated peptide*"). Hirokawa describes specificity to Fru-ValHis and Fru-Val for these two oxidase (see page 108, col. 1). Thus, the pH of 6.5 is used in the protease treatment (10 microliter volume: 5 µl of standard solution and 5 µl of protease), which was subsequently heat-inactivated and then diluted with the reaction mixture (140 µl) at a pH of 8.0 (see page 106). This combination of the two reactions, particularly where the reaction mixture constituted over 90% of the total reaction volume would have a pH very close, if not exactly, at 8.0 so when the oxidase is reacted with the released peptides, it would not be within the claimed range.

It is clear that the pH at which Hirokawa conducts the reaction with oxidase far exceeds the upper pH range in the claims (i.e., pH 7). Further, Hirokawa provides no disclosure for the adverse effects of fructosyl lysine and that such can be reduced by using the specific range of pH as is defined in the claims.

Hirokawa does not establish that one would have employed the pH range defined in the claims specifically in a method for reducing the effect of fructosyl lysine as claimed. Indeed, Hirokawa teaches that the optimum pH of the reaction with FPOX-E and -C was between 7.5 and 8.0 and that a pH below 5.5 significantly impaired enzymatic activity (see page 107, col. 1: "*Properties of recombinant FPOX-E*"). Again, referencing the

aforementioned discussion and Hirokawa's description of optimum pH in FIG. 1, these experiments were performed with the oxidase reacting with F-VH **but in the absence of fructosyl lysine compounds**. Further and notably. Hirokawa conducted reactions in acetate and Mes-NaOH buffer at a pH between 4 and 7 (see open triangles and squares in FIG. 1 of Hirokawa). Thus, this disclosure does not describe or suggest the claimed invention in which the effects of fructosyl lysine compound is reduced by conducting that reaction at a pH as defined in the claims.

Should the Examiner maintain that the Hirokawa provides sufficient motivation to modify the pH range to that which is claimed, Applicants presentation of comparative data is sufficient to rebut any such contention. As explained previously, the present invention has solved the problem of the conventional assays by reacting the enzyme at a pH of 4.0 to 7.0 with fructosyl valine or fructosyl valylhistidine released from the glycated protein and by measuring the product of the reacting at a pH of 4.0 to 7.0. The present inventors have found that the reactivity of the enzyme for assaying glycated protein with fructosyl lysine is decreased under the pH condition thereby the adverse effect of a fructosyl lysine compound on measuring a glycated protein is reduced. Prior to the present invention, nobody has found the effect of pH condition on the reactivity of the enzyme to fructosyl lysine nor reported to modify pH condition in order to reduce the adverse effect of fructosyl lysine on the assay of glycated protein. Specifically, in reference to the Examples in the present application, in Example 1, pH of 5.5, 6, 6.5 and 7 were compared to pH 7.5 and 8.0 (see page 19 of the present application). The results are presented in Table 1 and discussed in paragraph [0038] in the specification. Further demonstration of the importance of the claimed pH range is presented in Example 2, the results of which are presented in Table 2 on page 22 of the specification (noting that Table 2 was amended by preliminary amendment filed June 14, 2006). See also Example 3 starting at page 23, Example 4 starting at page 25 and Example 5

starting at page 28, each of which provide additional evidence as to the effects the improvements achieved when the reacting with the oxidase is conducted at the pH range defined in the claims. One of skill in the art would not have reasonably expected such an improvement, in terms of pH, based on the teachings provided in Hirokawa.

Therefore, the pH ranges coupled with the enzyme treatment for assaying fructosyl valine or fructosyl valylhistidine as defined in the claims reduce the effect a fructosyl lysine compound in assay of a glycated protein. Such is neither disclosed nor suggested by Hirokawa and as such withdrawal of the rejection is requested.

Upon consideration of the amendments and discussion submitted in this paper, a Notice of Allowance for all pending claims is also requested.

Respectfully submitted,

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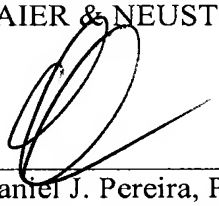

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